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TO ALL WHOM IT MAY CONCERN:

Be it known that I, SEO HONG YOO, a citizen of the United States, residing in Wyckoff, County of Bergen, State of New Jersey, whose post office address is 537 Spencer Drive, Wyckoff, NJ 07481, have invented an improvement in

PREPARATION OF AQUEOUS CLEAR SOLUTION DOSAGE FORMS WITH BILE ACIDS

of which the following is a

SPECIFICATION

This application claims the benefit of provisional application number 60/180,268, filed February 4, 2000 and is a continuation in part of application number 09/357,549 filed July 2, 1999 which claims the benefit of provisional application number 60/094,069, filed July 24, 1998, all of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Bile acids salts which are organic acids derived from cholesterol are natural ionic detergents that play a pivotal role in the absorption, transport, and secretion of lipids. In bile acid chemistry, the steroid nucleus of a bile acid salt has the

perhydrocyclopentano phenanthrene nucleus common to all perhydrosteroids. Distinguishing characteristics of bile acids include a saturated 19-carbon sterol nucleus, a beta-oriented hydrogen at position 5, a branched, saturated 5-carbon side chain terminating in a carboxylic acid, and an alpha-oriented hydroxyl group in the 3-position. The only substituent occurring in most natural bile acids is the hydroxyl group. In most mammals the hydroxyl groups are at the 3, 6, 7 or 12 positions.

The common bile acids differ primarily in the number and orientation of hydroxyl groups on the sterol ring. The term, primary bile acid refers to these synthesized *de novo* by the liver. In humans, the primary bile acids include cholic acid (3α, 7α, 12α-trihydroxy-5β-cholanic acid) ("CA") and chenodeoxycholic acid (3α, 7α-dihydroxy-5β-cholanic acid) ("CDCA") . Dehydroxylation of these bile acids by intestinal bacteria produces the more hydrophobic secondary bile acids, deoxycholic acid (3α, 12α-dihydroxy-5β-cholanic acid) ("DCA") and lithocholic acid (3α-hydroxy-5β-cholanic acid) ("LCA"). These four bile acids CA, CDCA, DCA, and LCA, generally constitute greater than 99 percent of the bile salt pool in humans. Secondary bile acids that have been metabolized by the liver are sometimes denoted as tertiary bile acids.

Keto-bile acids are produced secondarily in humans as a consequence of oxidation of bile acid hydroxyl groups, particularly the 7-hydroxyl group, by colonic bacteria. However, keto-bile acids are rapidly reduced by the liver to the corresponding α or β-hydroxy bile acids. For example, the corresponding keto bile acid of a CDCA is 7-keto lithocholic acid and one of its reduction products with the corresponding

β-hydroxy bile acid is ursodeoxycholic acid (3α-7β-dihydroxy-5β-cholanic acid) ("UDCA"), a tertiary bile acid.

UDCA, a major component of bear bile, has been used for the treatment of and the protection against many types of liver disease for a little over 70 years as a major pharmaceutical agent. Its medicinal uses include the dissolution of radiolucent gall stones, the treatment of biliary dyspepsias, primarily biliary cirrhosis, primary sclerosing choplangitis, chronic active hepatitis and hepatitis C. In other mammalian species, bile acids containing a 6β-hydroxyl group, which are found in rats and mice, are known as muricholic acid; 6α-hydroxy bile acids produced by swine are termed hyocholic acid and hyodeoxycholic acids. 23-hydroxy bile acids of aquatic mammals are known as phocecholic and phocedeoxycholic acids.

Typically, more than 99 percent of naturally occurring bile salts secreted into human bile are conjugated. Conjugates are bile acids in which a second organic substituent (e.g. glycine, taurine, glucuronate, sulfate or, rarely, other substituents) is attached to the side chain carboxylic acid or to one of the ring hydroxyl groups via an ester, ether, or amide linkage. Therefore, the ionization properties of conjugated bile acids with glycine or taurine are determined by the acidity of the glycine or taurine substituent.

Free, unconjugated, bile acid monomers have pK_a values of approximately 5.0. However, pK_a values of glycine conjugated bile acids are on average 3.9, and the pK_a of taurine conjugate bile acids are less than 1.0. The effect of conjugation, therefore, is to reduce the pK_a of a bile acid so that a large fraction is ionized at any given pH. Since the ionized salt form is more water soluble than the protonated acid form,

conjugation enhances solubility at a low pH. Free bile acid salts precipitate from aqueous solution at pH 6.5 to 7. In contrast, precipitation of glycine conjugated bile acid occurs only at pH of less than 5. Taurine conjugated bile acids remain in aqueous solution under very strongly acidic conditions (lower than pH 1). However, in the gastric pH range, certain bile acids such as UDCA and CDCA are no longer soluble.

Conjugation of the side chain of a bile acid with glycine or taurine has little influence on the hydrophobic activity of fully ionized bile salts. More hydrophobic bile salts exhibit greater solubilizing capacity for phospholipid and cholesterol and are consequently better detergents. More hydrophobic bile salts are also more injurious to various membranes, both *in vivo* and *in vitro*.

Natural bile salt pools invariably contain multiple bile acid salts. Mixtures of two or more bile salts of differing hydrophobic activity may behave as a single bile salt of an intermediate hydrophobic activity. As a result, detergent properties and the toxicity of mixtures of two bile acids of differing hydrophobic activity often are intermediate between the individual components. Biologic functions and biologic properties of bile acids resulting from their amphiphillic properties are as follows:

1. Ursodeoxycholic acid is a useful immuno-modulating agent.

2. Ursodeoxycholic acid inhibits deoxycholic acid-induced apoptosis by modulating mitochondrial transmembrane potential and reactive oxygen species production.

3. Ursodeoxycholic acid inhibits induction of nitric oxide synthase (NOS) in human intestinal epithelial cells and *in vivo*.

4. The hydrophilic nature of ursodeoxycholic acid confers cytoprotection in necroinflammatory diseases of the liver.

5. Ursodeoxycholic acid significantly improves transaminases and cholestatic enzymatic indices of liver injury in chronic hepatitis.

6. Bile acids substantially inhibit the growth of *H. pylori.*

7. Ursodeoxycholic acid is the most potent pepsin inhibitor among bile acids.

8. High levels of bile acids remarkably inhibit the proliferation of hepatitis C virus.

9. Ursodeoxycholic acid has cell membrane stabilizing properties.

10. Ursodeoxycholic acid alleviates alcoholic fatty liver.

11. Ursodeoxycholic acid has a vasodilative effect on the systemic vascular bed but altered neither pulmonary vascular function nor cardiac functions.

12. Bile acid synthesis from cholesterol is one of the two principal pathways for the elimination of cholesterol from the body.

13. Bile flow is generated by the flux of bile salts passing through the liver. Bile formation represents an important pathway for solubilization and excretion of organic compounds, such as bilirubin, endogenous metabolites, such as emphipathic derivatives of steroid hormones; and a variety of drugs and other xenobiotics.

14. Secretion of bile salts into bile is coupled with the secretion of two other biliary lipids, phosphatidylcholine (lecithin) and cholesterol. Coupling bile salt output with the lecithin and cholesterol output provides a major pathway for the elimination of hepatic cholesterol.

15. Bile salts, along with lecithin, solubilize cholesterol in bile in the form of mixed micelles and vesicles. Bile salt deficiency, and consequently reduced cholesterol solubility in bile, may play a role in the pathogenesis of cholesterol gallstones.

16. Bile acids are thought to be a factor in the regulation of cholesterol synthesis. At present, it is not certain whether they regulate the cholesterol synthesis by acting directly on the hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase or indirectly by modulating the cholesterol absorption in the intestine.

17. Bile salts in the enterohepatic circulation are thought to regulate the bile acid synthesis by suppressing or derepressing the activity of cholesterol 7-hydroxylase, which is the rate-limiting enzyme in the bile acid biosynthesis pathway.

18. Bile acids may play a role in the regulation of hepatic lipoprotein receptors (apo B.E.) and consequently may modulate the rate of uptake of lipoprotein cholesterol by the liver.

19. In the intestines, bile salts in the form of mixed micelles participate in the intraliminal solubilization, transport, and absorption of cholesterol, fat-soluble vitamins, and other lipids.

20. Bile salts may be involved in the transport of calcium and iron from the intestinal lumen to the brush border.

Recent drug delivery research concerning the characteristics and biofunctions of naturally occurring bile acid as an adjuvant and/or a carrier has focused on the derivatives and analogs of bile acids and bile acids themselves as novel drug delivery systems for delivery to the intestinal tract and the liver. These systems exploit the active transport mechanism to deliver drug molecules to a specific target tissue by oral or cystic administration. Thus, if bile acids or bile acid derivatives are rapidly and efficiently absorbed in the liver and, consequently, undergo enterohepatic cycling, many potential therapeutic applications are foreseen including the following: improvement of the oral absorption of an intrinsically, biologically active, but poorly absorbed hydrophillic and hydrophobic drug; liver site-directed delivery of a drug to bring about high therapeutic concentrations in the diseased liver with the minimization of general toxic reactions elsewhere in the body; and gallbladder-site delivery systems of cholecystographic agents and cholesterol gallstone dissolution accelerators. As an example, in 1985, Drs. Gordon & Moses et al. demonstrated that a therapeutically useful amount of insulin is absorbed by the nasal mucosa of human beings when administered as a nasal spray with common bile salts such as DCA, UDCA, CDCA, CA, TUDCA, and TCDCA. See Moses, Alan C., et al., Diabetes vol. 32 (November 1983) 1040-1047; Gordon, G.S., et al., Proc. Nat'l Acad. Sci. USA, vol. 82 (November 1985) 7419-7423.

In their experiment, bile acids produced marked elevations in serum insulin concentration, and about 50 percent decreases in blood glucose concentrations. However, this revolutionary nasal spray solution dosage form with bile acids (salts) as an adjuvant could not be developed further and commercialized, because the nasal spray solution must be prepared immediately prior to use due to the precipitation of bile acid salt and the instability of insulin at pH levels between 7.4 and 7.8. Moreover, as indicated in this disclosure, ursodeoxycholic acid as an adjuvant could not be used because of its insolubility at pH between 7.4 and 7.8.

Bile acid salts and insulin, thus, appear to be chemically and physically incompatible. The pH of commercial insulin injection solutions is between 2.5 and 3.5 for acidified dosage forms and is between 7.00 and 7.4 for neutral dosage forms. Dosage forms of bile acid salts prepared by conventional techniques have been unable to overcome problems with bile precipitation at these pH levels and insulin is unstable at a pH of 7.4 or higher. Therefore, safe and efficient preparations of any solution dosage forms of insulin with bile acid (salt) are not commercially available at this time.

Heparin, a most potent anticoagulant, is widely used in the treatment of and in the prevention of thromboembolism. However, heparin treatment is usually limited to hospitalized patients since this drug is given only by injection. Alternate routes which have been attempted are an intrapulmonary spray, suppositories, and enema. According to numerous publications, for heparin absorption through the gastrointestinal mucosa to be facilitated, the preparations should be in acidic condition. According to Dr. Ziv, Dr. Eldor et al., heparin was absorbed through the rectal mucosa of rodents and primates only when administered in solutions containing sodium cholate or sodium

deoxycholate. See Ziy E. et al., Biochemical Pharmacology, vol. 32, No. 5, pp. 773-776 (1983). However, heparin is only stable under acidic conditions. Bile acids are particularly not soluble in acidic conditions. Therefore, due to their incompatible characteristics, commercial dosage forms of bile acids with heparin are not presently available.

Drug delivery systems involving bile acids can provide liver-specific drug targeting which is of major interest for drug development since standard pharmacological approaches to liver diseases have been frustrated by the inadequate delivery of active agents into liver cells as well as non specific toxicity towards other organs. For example, the liver-specific delivery of a drug is necessary for inhibitors of collagen synthesis for the treatment for liver fibrosis in order to avoid unspecific and undesired side-effects in extrahepatic tissues. Furthermore, for the treatment of cancer of the biliary system, high drug levels must be achieved in the liver and the biliary system, whereas in extrahepatic tissues low drug concentrations are desired to minimize the cytoxicity of the cytostatics to normal non-tumor cells. Dr. Kramer, Dr. Wess et al. demonstrate that hybrid molecules formed by covalent linkages of a drug to a modified bile acid molecule are recognized by the Na+-dependent bile acid uptake systems in the liver and the ileum. See U.S. Patent No. 5,641,767. Even if bile acid salts and their derivatives act as shuttles for specific delivery of a drug to the liver, as already mentioned above, there are enormous risks to the development of the derivatives of bile acids or bile acid salts as carriers because new derivatives of bile acids or bile acid salts formed by covalent linkages of a drug to bile acid must be tested for its pharmacology, toxicity and clinical effectiveness. Thus, the development of preparations in which a drug can be absorbed with bile acids or bile acid

salts from the places which contain the excessive bile acids in the intestine is far easier and far more valuable than the development of the new bile acid derivatives because less testing is required.

In spite of the extremely valuable therapeutic activities and the long historic medical uses of bile acids as therapeutically active agents and as carriers and/or adjuvants based on the already mentioned biological properties and functions of bile acids, the commercial administration of bile acids is limited to pharmaceutical formulations with a solid form of bile acid which are in tablet, capsule and suspension. This is due to the insolubility of bile acid in aqueous media at pH from approximately 1 to 8. This is also due to bile's extremely bitter taste and equally bitter after-taste which lasts several hours. Ursodeoxycholic acid, chenodeoxycholic acid, and lithocholic acid are practically insoluble in water. Deoxycholic acid and cholic acid have solubilities of 0.24 g/L, and 0.2 g/L, respectively. Tauroursodeoxycholic acid, taurochenodeoxycholic acid, and taurocholic acid are insoluble in hydrochloric acid solution. The few aqueous dosage forms that are available are unstable, and have very limited uses because of pH control and maintenance problems. Moreover, some commercial pharmaceutical dosage forms of bile acids have been shown to have scant bioavailability as described in *European Journal of Clinical Investigation* (1985) 15, 171-178. Bile acid, especially ursodeoxycholic acid is poorly soluble in the gastro-duodeno jejunal contents of fasted subjects. From 21% to 50% of the ingested doses were recovered in solid form because of the unpredictable variations in the very slow progressive solubilization of solid ursodeoxycholic acid in the gastrointestinal track. Bile acids, particularly ursodeoxycholic acid, deoxycholic acid, chenodeoxycholic acid, cholic acid,

hyodeoxycholic acid, 7-keto lithcholic acid, tauroursodeoxycholic acid, and taurochenodeoxycholic acid among others, are especially insoluble in the gastric juices and in aqueous hydrochloric acid solution. However, the solubility of bile acids increase with the increase of the pH in the intestine very slowly and incompletely, and eventually the bile acids become soluble at pH between 8 and 9.5.

To overcome this slow and inefficient absorption process in the intestine due to the incomplete and slow solubilization of bile acids, many newly developed pharmaceutical formulations have been prepared, such as delayed release dosage forms with water soluble solid bile acids which are often strongly alkaline. These newly developed pharmaceutical dosage forms are enterosoluble-gastroresistant. These enterosoluble-gastroresistant dosage forms remain intact in gastric juices in the stomach, but are dissolved and release the strongly alkaline solid bile salts of the formulations at the targeted area, within a limited time once they reach the small intestine.

These types of dosage forms, of course, showed better bioavailability than presently commercialized dosage forms as described in U.S. Patent No. 5,380,533. However, it is extremely difficult and very costly to prepare the precise delayed release dosage forms which can release therapeutically active components by disintegration, dissolution and diffusion at the desired area within a limited time. According to U.S. Patent No. 5,302,398, the absorption test of the gastroresistant enterosoluble dosage forms of bile acids, particularly ursodeoxycholic acid in man show that its absorption increases a value of about 40 percent in comparison with administering the same amount in current commercial dosage forms. Its maximum hematic concentrations are on average three times higher, and are reached faster than with the commercial formulations. Any

dosage forms of bile acid formula must be capable of releasing bile acids in a known and consistent manner following administration to the patient. Both the rate and the extent of release are important, and should be reproducible. Ideally, the extent of release should approach 100 percent, while the rate of release should reflect the desired properties of the dosage form.

It is a well-known fact that solution dosage forms of drugs show significantly improved rates and extents of absorption, compared to the same drug formulated as a tablet, capsule, or suspension. This is because solution dosage forms are chemically and physically homogeneous solutions of two or more substances. Moreover, the specially designed solution dosage forms which can maintain the solution systems without breaking down under any pH conditions are ready to be diffused in the desired area for immediate and complete absorption, whereas tablets, capsules or delayed release formulations must invariably undergo disintegration, dissolution and diffusion at the desired area within a limited time. Unpredictable variations in the extent and rate of release of bile acids by the disintegration, dissolution and diffusion of delayed or immediate release dosage forms having pH-dependent instability result in the slow and inefficient bile absorption and reduced bioavailability.

The luminal surface of the stomach is coated with a thick layer of protective mucus. The mucus gel coating maintains a pH gradient from the intraluminal compartment to the apical membrane and is believed to contribute to the phenomenon of cytoprotection. *H. pylori* infection occurs on the luminal surface of the stomach mucosa within the mucus, on the epithelial surface, and within the gastric pints. Bacterial enzymes are believed to degrade the mucus glycoprotein network and reduce the

polymers to monomers (or subunits) such that the mucus can no longer exist as a gel. In addition, mucinogenesis is reduced and the mucosa becomes susceptible to the erosive effects of acid. This condition may lead to gastritis and peptic ulcers.

Bismuth compounds have gained increasing interest in the therapeutic treatment of gastro-duodenal disorders and especially in the eradication of *Helicobacter pylori*, a bacterium thought to be involved in the etiology of the disease. Many oral preparations of bismuth have been used. The various preparations appear to differ in clinical efficacy as well as pharmacokinetics. The inorganic salts used have included subnitrate, subcarbonate, subgallate, tartarate, citrate and subsalicylate. The commercial preparations have generally been available over the counter and have often contained other compounds in addition to the bismuth salt. The commercial preparations have been used successfully in the treatment of both gastric and duodenal ulcer disease. These preparations have proved as effective as the histamine H2 antagonists in the treatment of gastric and duodenal ulcers and have been associated with lower relapse rates after cessation of therapy. The lower relapse rate after initial healing with bismuth preparations have been attributed to its ability to eradicate *H. pylori* and to moderate the gastroduodenitis associated with infection by this organism. Long-term eradication of *H. pylori* is more likely when bismuth preparation is administered along with antibiotics or antiseptics (local delivery) such as bile acids.

A variety of antibiotics and antiseptics display good activity against *H. pylori in vitro*. Yet when tested as single agents in clinical studies, they do not succeed in eradicating the organism. Failure of therapy and relapse are very common. The reason for this discrepancy between *in vitro* and clinical results has not been established. Possible

explanations are poor penetration of the compounds into gastric mucus, destruction at acid pH, insolubility in acidic environment, and combinations thereof. Consequently, administration of high doses of antimicrobial agents on a daily basis is necessary for *H. pylori* eradication. The efficacy of this course of therapy is hindered by poor patient compliance due to adverse effects such as diarrhea, nausea, retching and breakdown of normal intestinal flora.

Another reason for incomplete eradication may be that the residence time of antimicrobial agents in the stomach is so short that effective antimicrobial concentrations cannot be achieved in the gastric mucous layer or epithelial cell surfaces where *H. pylori* exist. Therefore, eradication of *H. pylori* may be better achieved by a therapy that improves antimicrobial agent delivery for topical activity and absorption for systemic activity. However, no *in vivo* eradication trials with dosage forms that prolong the gastric residence times for topical activity and have the high absorption rate in the gastro-intestinal tract have been reported. The best results so far have been achieved with the combination of a non-absorbed agent with topical activity, colloidal bismuth compounds, and a well-absorbed agent with systemic activity, amoxicillin (Van Caekemberghe and Breyssens, 1987, *Antimicrobial Agent and Chemotherapy*, pp 1429-1430). But clearly, therapy for *H. pylori* infections is still suboptimal.

In addition to a bactericidal effect, some bismuth compounds have profound effects on some of the pathogenic mechanisms whereby *H. pylori* damage the mucosa. Bismuth compounds are potent and non-specific enzyme inhibitors. *In vitro* studies have suggested that these compounds may inhibit bacterial enzymes, including lipases, proteases, and glycosidases synthesized by *H. pylori*. Inhibition of bacteria

enzymes and maintenance of an intact viscoelastic gel coating is thought to be related to the therapeutic action of bismuth compounds for *H. pylori* associated gastritis and peptic ulcers.

Bismuth compounds block the adhesion of *H. pylori* to epithelial cells. Shortly after oral administration of these compounds, the organisms were located inside, rather than underneath, the mucus gel. This was thought to result from loss of adherence to the apical membrane of the gastric epithelial cells. Bismuth, in the form of electron dense bodies, was seen to be deposited on the surface and within the bacterial cell. But unfortunately, intramucus bismuth concentrations often fall below the mean inhibitory concentration of bismuth compounds for *H. pylori*, because of the diluting effects of food, long disintegration time of commercial tablets at pH<1.1, and bismuth precipitation due to insolubility in acidic environment. Additionally, *H. pylori* inactivated by exposure to growth-inhibiting concentrations of bismuth compounds can remain viable for several hours and, therefore is capable of resuming normal growth when bismuth is removed.

The inhibitory factor(s) in bile was markedly reduced by acidification followed by centrifugation to remove precipitated glycine-conjugated bile acids and was completely eliminated by use of the bile acid- sequestering agent cholestyramine. These results are consistent with the notion that acidic conditions in the duodenal bulb would serve to precipitate inhibitory bile acids (or other inhibitory substances) and allow *H. pylori* to grow in an otherwise hostile environment. The observations related to reflux gastritis by D.Y. Graham (Osato et al., 1999, Digestive Diseases and Sciences 44(3):462-464) can be extended to possibly understand the relation between acid secretion and duodenal ulcer. Low pH in the duodenal bulb in patients with duodenal ulcer disease

associated with high acid secretion, rapid gastric emptying, and local production of acid would both promote development of gastric metaplasia and precipitate the deleterious glycine-conjugated bile acids, allowing *H. pylori* to colonize and thrive.

SUMMARY OF THE INVENTION

The present invention relates to a composition which comprises (1) a bile acid, its derivative, its salt, or its conjugate with an amine, (2) water, and (3) a sufficient quantity of an aqueous soluble starch conversion product such that the bile acid and the starch conversion product remain in solution at any pH within a selected pH range.

The invention further relates to a composition which comprises (1) a bile acid, its derivative, its salt, or its conjugate with an amine, (2) water, and (3) a sufficient quantity of an aqueous soluble non-starch polysaccharide such that the bile acid and the polysaccharide remain in solution at any pH within a selected pH range.

The invention further relates to a pharmaceutical composition which comprises (1) a bile acid, its salt, or its conjugate with an amine, (2) water, (3) a pharmaceutical compound in a pharmaceutically appropriate amount, and (4) a sufficient quantity of an aqueous soluble starch conversion product or an aqueous soluble non-starch polysaccharide such that the bile acid, the pharmaceutical compound, and the carbohydrate remain in solution at any pH level within a selected pH range.

The invention further relates to solution dosage forms of bile acid compositions. Advantages of these solution dosage forms include improved bioavailability and absorbability of a bile acid. Additional advantages of solution dosage forms include improved bioavailability and absorbability of a pharmaceutical compound.

In some embodiments of the invention, a composition is provided which comprises (1) a bile acid, its derivative, its salt, or its conjugate with an amine, (2) water, and (3) a sufficient quantity of carbohydrate such that the bile acid component and the carbohydrate remain in solution at any pH within a selected pH range, wherein the carbohydrate is a combination of an aqueous soluble starch conversion product and an aqueous soluble non-starch polysaccharide. In embodiments containing both soluble non-starch polysaccharide and high molecular weight starch conversion product, the amounts of each are such that when combined together in the composition they are sufficient to allow the bile acid component, the high molecular weight starch conversion product, the soluble non-starch polysaccharide and the pharmaceutical compound, if any, to remain in solution at any pH within a selected pH range.

In some embodiments of the invention, a combination therapy composition is provided which may increase intensity of response to or efficacy of a pharmaceutical. Such a composition may permit administration of lower dosages of a pharmaceutical compound, attack a disease complex at different points, affect elimination and/or alter absorption of a pharmaceutical compound. Such a composition may lead to or contribute to a reduction in toxicity and/or side effects of a pharmaceutical.

The invention further relates to a composition which comprises (1) a bile acid, its salt, or its conjugate with an amine, (2) water, (3) an aqueous soluble bismuth compound, and (4) a sufficient quantity of an aqueous soluble starch conversion product or an aqueous soluble non-starch polysaccharide such that the bile acid, such that the bile acid, bismuth, and carbohydrate remain in solution at any pH level within a selected pH range.

The invention further relates to a method of treating or preventing a human or animal disease comprising administration of a composition of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Graph of blood serum - concentration of UDCA (squares) and GUDCA (triangles) versus time following administration of dosage formulations according to Examples II and VI and Table 4.

Figure 2: Graph of blood serum concentration of UDCA versus time following administration of dosage formulations of the bile acid according to Examples III and VI and Table 4.

Figure 3: Diagram of the mean(n=5) for group I for pharmacokinetic parameters of UDCA in human after an oral administration of liquid formulation of UDCA prepared according to Example IX without bismuth.

Figure 4: Diagram of the mean(n=5) for group II for pharmacokinetic parameters of UDCA in human after an oral administration of liquid formulation of UDCA prepared according to Example IX.

Figure 5A. Transmission electron micrograph of *H. pylori* cultured from Columbia medium.

Figure 5B. Transmission electron micrograph of *H. pylori* 48 hrs after being treated with UDCA & bismuth citrate prepared according to Example IX.

Figure 5C. Transmission electron micrograph of *H. pylori* 72 hrs after being treated with UDCA & bismuth citrate.

Figure 6: NMR data for UDCA in a liquid formulation dosage form prepared according to Example III without preservatives, flavoring agent, and sweetener.

Figure 7: HPLC trace of UDCA in a liquid formulation dosage form prepared according to Example III without preservatives, flavoring agent, and sweetener.

Figure 8: HPLC trace of a UDCA standard.

Figure 9: *H. pylori* culture method.

Figure 10: *H. pylori* culture method.

Figure 11: *H. pylori* culture method.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an aqueous solution comprising (i) one or more soluble bile acids, aqueous soluble bile acid derivatives, bile acid salts, or bile acid conjugated with an amine, (collectively "bile acid"), (ii) water, and (iii) one or more aqueous soluble starch conversion products or aqueous soluble non-starch polysaccharide in an amount sufficient to produce a solution which does not form a precipitate at any pH within a desired pH range. In a preferred embodiment of the invention, the bile acid and the carbohydrate do not precipitate between about pH 1 and about pH 10, more preferably between about pH 1 and about pH 14, and most preferably at all pH values obtainable in an aqueous system. In some embodiments of the invention, a bile acid remains dissolved under acidic conditions as a free bile acid in spite of the general insolubility of bile acids under acidic conditions. In some embodiments of the invention, the composition may be used as a pharmaceutical formulation wherein the pharmaceutical compound remains in

solution without precipitation at prevailing pH levels in the mouth, stomach and intestines. The composition may contain a bile acid or its salt which itself has pharmaceutical effectiveness. Formulations of the invention may act as a carrier, an adjuvant or enhancer for the delivery of a pharmaceutical material which remains dissolved in the composition of the invention across the desired pH range. In some embodiments of the invention, a non-bile acid pharmaceutical is used though not in solution.

It is an advantage of this invention that the bile acid and the carbohydrate remain in solution without precipitation at any pH from acidic to alkaline. These aqueous solution systems of bile acid are substantially free of precipitate or particles. A further advantage of this invention is that the aqueous solution systems demonstrate no changes in physical appearance such as changes in clarity, color or odor following the addition of strong acids or alkali even after several months observation under accelerated conditions of storage at 50°C.

In some embodiments of the invention, an aqueous solution system of bile acid is administered orally whereupon it reaches the intestine through the gastrointestinal track without precipitation of bile acids as solids by exposure to acidic gastric juices and alkaline juices of the intestine. These dissolved bile acid formulations demonstrate intact solution systems in the intestine can be effectively and completely absorbed and, consequently, undergo enterohepatic cycling. According to the invention, bile acid solubility (e.g. precipitation and changes in physical appearance) is unaffected by whether a carboxylic acid side chain of certain bile acids can be protonated (non-ionized), ionized, or a simple carboxylic acid.

The ionization state of a bile acid carboxylic acid side chain greatly effects the hydrophobicity and the hydrophillicity of the bile acid in these aqueous solution systems. In some embodiments of the invention, that ionization state is manipulated by adjusting the pH to control the toxicity, absorption, and amphiphilicity of bile acids. One or more bile acid may be dissolved in these aqueous solution systems as a therapeutically active agent, as an adjuvant of a drug, as a carrier of drug or as an enhancer of drug solubility. These aqueous solution systems may be prepared for oral consumption, enemas, mouthwashes, gargles, nasal preparations, otic preparations, injections, douches, topical skin preparations, other topical preparations, and cosmetic preparations which have a desired pH without the disadvantage of precipitation or deterioration in physical appearance after long periods of time.

Soluble bile acids are any type of aqueous soluble bile acids. A bile acid salt is any aqueous soluble salt of a bile acid. The soluble bile acid derivatives of this invention are those derivatives which are as soluble or more soluble in aqueous solution than is the corresponding underivatized bile acid. Bile acid derivatives include, but are not limited to derivatives formed at the hydroxyl and carboxylic acid groups of the bile acid with other functional groups including but not limited to halogens and amino groups. Aqueous dissolved salts of bile acids may be formed by the reaction of bile acids described above and an amine including but not limited to aliphatic free amines such as trientine, diethylene triamine, tetraethylene pentamine, and basic amino acids such as arginine, lysine, ornithine, and ammonia, and amino sugars such as D-glucamine, N-alkylglucamines, and quaternary ammonium derivatives such as choline, heterocyclic amines such as piperazine, N-alkylpiperazine, piperidine, N-alkylpiperidine, morpholine,

N-alkylmorphline, pyrrolidine, triethanolamine, and trimethanolamine. According to the invention, aqueous soluble metal salts of bile acids, inclusion compound between the bile acid and cyclodextrin and its derivatives, and aqueous soluble O-sulfonated bile acids are also included as soluble bile acid salts. Soluble bile acid may include an aqueous preparation of a free acid form of bile acid combined with one of HCl, acetic acid, ammonia, or arginine.

Bile acids used in this invention include, but are not limited to ursodeoxycholic acid, chenodeoxycholic acid, cholic acid, hyodeoxycholic acid, deoxycholic acid, 7-oxolithocholic acid, lithocholic acid, iododeoxycholic acid, iocholic acid, tauroursodeoxycholic acid, taurochenodeoxycholic acid, taurodeoxycholic acid, taurolithocholic acid, glycoursodeoxycholic acid, taurocholic acid, glycocholic acid, and their derivatives at a hydroxyl or carboxylic acid group on the steroid nucleus.

A major advantage of the instant invention is that by delivery of bile acid in solution, it achieves higher *in vivo* levels of bile acids than conventional preparations. Therefore, the therapeutic potential of bile acid may be more fully acheived than previous formulations. The *in vivo* levels of bile acids attainable with existing formulations in which bile is incompletely solubilized are lower and require administration of larger amounts of bile acids. Since bile acid is completely dissolved in the inventive formulations, higher *in vivo* levels of bile acid may be achieved, even though lower doses are administered.

In some embodiments of the invention, a plurality of bile acids are used in a single formulation. Mixtures of two or more bile salts of differing hydrophobic activity may behave as a single bile salt of an intermediate hydrophobic activity. As a result,

detergent properties and the toxicity of mixtures of two bile acids of differing hydrophobic activity often are intermediate between the individual components.

Carbohydrates suitable for use in the invention include aqueous soluble starch conversion products and aqueous soluble non-starch polysaccharides. For purposes of the invention, aqueous soluble starch conversion products are defined as follows:

1. They may be obtained under various pH conditions from the partial or incomplete hydrolysis of starch.
2. Non-limiting examples include maltodextrin, dextrin, liquid glucose, corn syrup solid (dried powder of liquid glucose), soluble starch, and soluble starch, preferably maltodextrin or corn syrup solid, most preferably corn syrup solid. Particularly preferred are MALTRIN® M200, a corn syrup solid, and MALTRIN® M700 a maltodextrin, both of which are manufactured by GPC®, Grain Processing Corporation of Muscatine, Iowa. For the purpose of this invention, the term "corn syrup" includes both corn syrup and liquid glucose.
3. If polymeric, the polymer has at least one reducing end and at least one non-reducing end. The polymer may be linear or branched.
4. The molecular weight is from about 100 mass units to over 10^6 mass units. High molecular weight aqueous soluble starch conversion products are those having a molecular weight over 10^5.

For purposes of the invention, aqueous soluble non-starch polysaccharides are defined as follows:

1. They may be obtained under various pH conditions by various hydrolytic or synthetic mechanisms.
2. Non-limiting examples include to dextran, guar gum, pectin, indigestible soluble fiber.
3. If polymeric, the polymer has at least one reducing end and at least one non-reducing end. The polymer may be linear or branched.
4. The molecular weight is from about 100 mass units to over 10^6 mass units. Preferably the molecular weight is over 10^5 mass units.

The amount of high molecular weight aqueous soluble starch conversion product and/or soluble non-starch polysaccharide used in the invention is at least the amount needed to render the chosen bile acid salt soluble in the concentration desired and in the pH range desired. In preferred embodiments of the invention, the approximate minimal quantity of maltodextrin required to prevent the precipitation of bile acids from the aqueous solution dosage forms of the invention is 5 g for every 0.2 g of ursodeoxycholic acid, 25 g for every 1g of ursodeoxycholic acid, and 50 g for every 2 g of ursodeoxycholic acid in 100 mL of water. In preferred embodiments of the invention, the approximate minimal quantity of maltodextrin is 30 g for every 200 mg of chenodeoxycholic acid, 12 g for every 200 mg of 7-ketolithocholic acid, 10 g for every 200 mg of cholic acid and 50 g for every 200 mg of deoxycholic acid. In preferred embodiments of the invention, the approximate minimal quantity of liquid glucose

(commercial light corn syrup) required to prevent the precipitation of bile acids from the aqueous solution dosage forms of the invention is 80 g for every 500 mg ursodeoxycholic acid in 100 mL water, and 80 g for every 500 mg ursodeoxycholic acid in 200 mL water. In preferred embodiments of the invention, the approximate minimal quantity of dried powder of liquid glucose (corn syrup solid, e.g., MALTRIN® M200) required to prevent the precipitation of bile acids from the aqueous solution dosage forms of the invention is 30 g for every 500 mg ursodeoxycholic acid in 100 mL water, and approximately 30 g for every 500 mg of ursodeoxycholic acid in 200 mL water. In preferred embodiments of the invention, the approximate minimal quantity of soluble non-starch polysaccharide (e.g., pectin, guar gum, gum arabic) required to prevent the precipitation of bile acids from the aqueous solution dosage forms of the invention is 50 g guar gum for every 500 mg ursodeoxycholic acid in 100 mL water and 80g of pectin for every 500 mg of ursodeoxycholic acid in 100 mL water. The minimal required quantity of high molecular weight aqueous soluble starch conversion products or soluble non-starch polysaccharide is primarily determined by the absolute quantity of bile acids in the solution formulation rather than the concentration.

In some embodiments of the invention, a formulation may comprise cyclodextrin.

In some embodiments of the invention, the formulation further comprises dietary fiber. Non-limiting examples of dietary fiber include guar gum, pectin, psyllium, oat gum, soybean fiber, oat bran, corn bran, cellulose and wheat bran.

In some embodiments of the invention, the formulation further comprises emulsifying agents. For the purpose of the invention, the term “emulsifying agent”

includes emulsifying agents and suspending agents. Non-limiting examples of emulsifying agents include guar gum, pectin, acacia, carrageenan, carboxymethyl cellulose sodium, hydroxymethyl cellulose, hydroxypropyl cellulose, methyl cellulose, polyvinyl alcohol, povidone, tragacanth gum, xanthan gum, and sorbitan ester.

The selected pH range for which the formulation will not precipitate its bile acid, starch conversion product, soluble non-starch polysaccharide or its pharmaceutical compound may be any range of pH levels obtainable with an aqueous system. Preferably this range is between about pH 1 and about pH 14 and more preferably between about pH 1 and about pH 10. Still more preferably the range is any subset of the range of pH levels obtainable in an aqueous system sufficient for the pharmaceutical formulation to remain in solution from preparation, to administration, to absorption in the body, according to the method of administration.

The invention contemplates the use of a broad range of pharmaceutical compounds. Non-limiting examples include hormones, hormone antagonists, analgesic, antipyretics, antiinflammatory drugs, immunoactive drugs, antineoplastic drugs, antibiotics, anti-inflammatory agents, sympathomimetic drugs, anti-infective drugs, anti-tumor agents, and anesthetics. Further non-limiting examples include drugs that target or effect the gastrointestinal tract, liver, cardiovascular system, and respiratory system. Further non-limiting examples of pharmaceutical compounds include insulin, heparin, calcitonin, ampicillin, octreotide, sildenafil citrate, calcitriol, dihydrotachysterol, ampomorphine, yohimbin, trazodone, acyclovir, amantadine·HCl, rimantadine·HCl, cidofovir, delavirdine·mesylate, didanosine, famciclovir, forscarnet sodium, fluorouracil, ganciclovir sodium, idoxuridine, interferon-α, lamivudine, nevirapine, penciclovir,

ribavirin, stavudine, trifluridine, valacyclovir·HCl, zalcitabine, zidovudine, indinavir·H_2SO_4, ritonavir, nelfinavir·CH_3SO_3H, saquinavir·CH_3SO_3H, d-penicillamine, chloroquine, hydroxychloroquine, aurothioglucose, gold sodium thiomalate, auranofin levamisole, DTC, isoprinosine, methyl inosine monophosphate, muramyl dipeptide, diazoxide, hydralazine·HCl, minoxidil, dipyridamole, isoxsuprine·HCl, niacin, nylidrin·HCl, phentolamine, doxazosin·CH_3SO_3H, prazosin·HCl, terazocin·HCl, clonidine·HCl, nifedipine, molsidomine, amiodarone, acetylsalicylic acid, verapamil, diltiazem, nisoldipine, isradipine, bepridil, isosorbide·dinitrate, pentaerythrytol·tetranitrate, nitroglycerin, cimetidine, famotidine, nizatidine, ranitidine, lansoprazole, omeprazole, misoprostol, sucralfate, metoclopramide·HCl, erythromycin, bismuth compound, alprostadil, albuterol, pirbuterol, terbutaline·H_2SO_4, salmetrol, aminophylline, dyphylline, ephedrine, ethylnorepinephrine, isoetharine, isoproterenol, metaproterenol, n·docromil, oxy triphylline, theophylline, bitolterol, fenoterol, budesonide, flunisolide, beclomethasone·dipropionate, fluticasone·propionate, codeine, codeine sulfate, codeine phosphate, dextromethorphan·HBr, triamcinolone·acetonide, montelukast sodium, zafirlukast, zileuton, cromolyn sodium, ipratropium bromide, nedocromil sodium benzonate, diphenhydramine·HCl, hydrocodone·bitartarate, methadone·HCl, morphine sulfate, acetylcysteine, guaifenesin, ammonium carbonate, ammonium chloride, antimony potassium tartarate, glycerin, terpin·hydrate, colfosceril palmitate, atorvastatin·calcium, cervastatin·sodium, fluvastatin·sodium, lovastatin, pravastatin·sodium, simvastatin, picrorrhazia kurrva, andrographis paniculata, moringa oleifera, albizzia lebeck, adhata vasica, curcuma longa, momordica charantia, gymnema sylvestre, terminalia arjuna, azadirachta indica, tinosporia cordifolia, metronidazole,

amphotericin B, clotrimazole, fluconazole, haloprogin, ketoconazole, griseofulvin, itraconazole, terbinafin·HCl, econazole·HNO_3, miconazole, nystatin, oxiconazole·HNO_3, sulconazole·HNO_3, cetirizine·2HCl, dexamethasone, hydrocortisone, prednisolone, cortisone, catechin and its derivatives, glycyrrhizin, glycyrrhizic acid, betamethasone, ludrocortisone·acetate, flunisolide, fluticasone·propionate, methyl prednisolone, somatostatin, lispro, glucagon, proinsulin, insoluble insulins, acarbose, chlorpropamide, glipizide, glyburide, metformin·HCl, repaglinide, tolbutamide, amino acid, colchicine, sulfinpyrazone, allopurinol, piroxicam, tolmetin sodium, indomethacin, ibuprofen, diflunisal, mefenamic acid, naproxen, and trientine.

Additional pharmaceutical compounds that may be included in the formulation are any compounds which remain soluble when added to the formulation. With an additional pharmaceutical compound in the formulation, a bile acid in solution may act as an adjuvant, carrier, or enhancer for the solubility of certain therapeutically active agents, including, but not limited to, insulin (pH 7.4-7.8), heparin (pH 5-7.5), calcitonin, ampicillin, amantadine, rimantadine, sildenafil, neomycin sulfate (pH 5-7.5), apomorphine, yohimbin, trazodone, ribavirin, paclitaxel and its derivatives, retinol, and tretinoin, which are soluble and stable in acid and/or alkali and can be added as needed into these aqueous solution dosage forms of certain concentrations of bile acids in this invention. Certain therapeutically active agents, including, but not limited to, metformin HCl (pH 5-7), ranitidine HCl, cimetidine, lamivudine, cetrizine 2HCl (pH 4-5), amantadine, rimantadine, sildenafil, apomorphine, yohimbine, trazodone, ribavirin and dexamethasone, hydrocortisone, prednisolone, triamcinolone, cortisone, niacin, taurine, vitamins, naturally occurring amino acids, catechin and its derivatives, glycyrrhizal

extract and its main constituents such as glycyrrhizin and glycyrrhizic acid, water soluble bismuth compounds (e.g., bismuth sodium tartrate), and which are soluble and stable in acid and/or alkali can be added as needed into these aqueous solution dosage formulations containing ursodeoxycholic acid in this invention.

According to the invention bismuth compounds comprise an aqueous soluble reaction product between a bismuth ion and a chelator. Non-limiting examples of such chelators include citric acid, tartaric acid, malic acid, lactic acid and eidetic acid and alkalies. Non-limiting examples of bismuth compounds include an ammonium salt of a bismuth chelation complex, bismuth citrate, bismuth gallate, bismuth sulphate, bismuth subnitrate, bismuth subsalicylate, tripotassium dicitrato bismuthate, and bismuth sodium tartrate.

The invention contemplates the use of pH adjustable agents. Non-limiting examples include HCl, H_2SO_4, HNO_3, CH_3COOH, citric acid, malic acid, tartaric acid, lactic acid, phosphate, eidetic acid and alkalies.

In some embodiements of the invention, the formulations may be used to treat human and mammalian diseases. The invention contemplates treating gastrointestinal disorders, liver diseases, gall stones, and hyperlipidemia. Non-limiting examples of liver diseases include alcohol-induced liver diseases and non-alcohol-induced liver diseases. Non-limiting examples of gastrointestinal disorders include chronic gastritis, reflux gastritis, and peptic ulcer disease. Non-limiting examples of non-alcohol-induced liver diseases include primary biliary cirrhosis, acute and chronic hepatitis, primary sclerosing cholangitis, chronic active hepatitis, and excess accumulation of fat in the liver. The invention further contemplates treating viral,

bacterial and fungal diseases. In some embodiments of the invention, a formulation is administered to treat and/or erradicate *Helicobacter pylori* infection. In some embodiments of the invention, a formulation is administered to treat and/or erradicate hepatitis C virus infection, influenza A, Influenza C, parainfluenza 1, sendai, rubella, and pseudorabies virus. In some embodiments of the invention, a formulation is administered to treat acute or chronic inflammatory diseases. Non-limiting examples of inflammatory diseases include bronchitis, chronic pharyngitis, and chronic tonsillitis. In some embodiments of the invention, a formulation is administered to treat hypercholersterolemia.

In some embodiments of the invention, the formulation is modified such that it may be administered as a liquid, solid, powder or tablet. In some embodiments of the invention, the formulation is comprised in a syrup, thick syrup or paste. A non-limiting example of a syrup is a solution of maltodextrin wherein the concentration of maltodextrin is less than 1.0 kg/L. A non-limiting example of a thick syrup is a solution of maltodextrin wherein the concentration of maltodextrin is between 1.0 kg/L and 1.2 kg/L inclusive. A non-limiting example of a paste is a solution of maltodextrin wherein the concentration of maltodextrin is greater than 1.2 kg/L.

EXAMPLES

The stability of dosage formulations of the invention were evaluated by measuring the concentration of the relevant bile acid over time in preparations comprising soluble bile acid, a high molecular weight aqueous soluble starch conversion product, and water at various pH and temperature levels. The retention time of each bile acid may be

adjusted as needed to permit individual analysis each bile acid present in a complex samples, i.e. a sample having a plurality of bile acids.

The stability tests were conducted on three different aqueous solution systems:

1. A bile acid and a high molecular weight aqueous soluble starch conversion product were combined in aqueous solution according to Example I, with results as shown in Tables 1A and 1B.
2. Mixed bile acids and high molecular weight aqueous soluble starch conversion products were combined in aqueous solution according to Example II, with results as shown in Table 2 .
3. Bile acids, high molecular weight aqueous soluble starch conversion products and branched chained amino acids (e.g. leucine, isoleucine, valine, or other amino acid with a branched side chain) were combined in aqueous solution according to Example IV, with results as shown in Tables 3A through 3F.

The stability tests were performed with HPLC and microscope light at various pH conditions under the normal and accelerated conditions. Accelerated conditions for testing pharmaceutical compositions have been described (Remington, *The Science and Practice of Pharmacy*, 19th ed., p. 640). All of these stability test results were satisfactory in that the concentration of bile acid as measured by HPLC did not change appreciably over time at various pH levels. Thus, the formulations of the examples are suitable for preparing a commercial liquid dosage form. Particularly, all solution formulations which contained bile acid showed excellent results in the stability

tests with no precipitation and no physical appearance changes over the test period. Some formulations remain stable for over 2 years.

Moreover, the solution stability tests were conducted on the aqueous solution dosage forms comprising the mixture of aqueous soluble UDCA, branched chained amino acid (leucine, isoleucine, valine) and maltodextrin according to example IV as a typical example of the solution dosage forms in which bile acid as a therapeutically active agent, as an adjuvant or carrier, pharmaceutically active agent, or enhancer of solubility, and high molecular weight aqueous soluble starch conversion products or soluble non-starch polysaccharides are dissolved. According to the test results, there is no discoloration, no clarity changes, and no precipitation. Furthermore, there are no detectable impurities from the deterioration of UDCA or branched chained amino acids when examined by HPLC at various pH conditions such as pH 1, 3, 5, 7, 9, and 10 under the accelerated conditions or incubation at (50°C).

The aqueous solution dosage forms according to this invention did not change either physically or chemically at various pH conditions under the accelerated conditions despite the addition of therapeutically and chemically active agents that are stable and soluble in hydrochloric acid solution. Therefore, these aqueous solution systems are extremely valuable pharmaceutical dosage forms for the therapeutically active bile acids preparations, and/or the drug (pharmaceutical compound) delivery preparations in which bile acids play roles as the adjuvant of drug, the carrier of drug, or the enhancer of solubility of a drug by micelle formation at various pH conditions without the stability problems, including precipitation in acidic conditions.

For the solution stability test for each bile acid, HPLC was used to measure the concentration of the relevant soluble bile acid under the following conditions: the elution solvent of 0.02 M KH_2PO_4:acetonitrile in a ratio of 55:45, with a pH of 3.01, the flow rate was 0.8 mL/min., the injection volume was 20 μL, wave length for detection was 195 nm. In the tables, the concentration of the indicated bile acid salt for each of the three numbered trials and the average thereof is reported on each line. The percentage indicates the relative concentration of the bile acid salt after incubation for a certain amount of time in comparison with the initial concentration.

Example I

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH tested.

Soluble bile acid	200 mg (as free acid)
Minimal quantity of maltodextrin	for CDCA: approx. 30 g;
	for UDCA: approx. 5 g;
	for 7-ketolithocholic acid: approx. 12 g;
	for cholic acid: approx. 10 g;
	for deoxycholic acid: approx. 50 g;
	for hyodeoxycholic acid: approx. 3.5 g
Purified water to make	100 mL

100 mL of the aqueous solution in which one of the above bile acids is dissolved was prepared. Into the resulting clear solution, maltodextrin, a high molecular weight aqueous soluble starch conversion product, was added with agitation at room temperature. Purified water was added to adjust the total volume to be 100 mL. According to the instant invention and all examples, purified water is deionized, distilled deionized-distilled water, or a grade commonly used for pharmaceutical preparations.

Based on these formulas, the aqueous solution dosage forms of various concentrations of certain bile acids (or salts) with its corresponding minimal quantity or more of high molecular weight aqueous soluble starch conversion products (for example; maltodextrin, liquid glucose, dried powder of liquid glucose (commercial corn syrup solid), dextran, dextrin, and soluble starch) or soluble non-starch polysaccharide (e.g. guar gum, pectin, gum arabic) were prepared.

Example II

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH tested.

Soluble cholic acid	200 mg (as free acid),
Soluble 7-ketolithocholic acid	200 mg (as free acid),
Soluble chenodeoxycholic acid	200 mg (as free acid),
Minimal quantity of maltodextrin	40 g, and
Purified water to make	100 mL

60 mL of the aqueous solution in which soluble cholic acid, soluble 7-ketolithocholic acid, and soluble chenodeoxycholic acid are dissolved, was prepared.

Into the resulting clear solution, maltodextrin was added with agitating at room temperature. Purified water was added to adjust the total volume to be 100 mL.

Using this formulation, the stability test for the aqueous solution of the mixture of various bile acids which can control the hydrophillicity or hydrophobicity was conducted.

Table 1A shows the results of a test of stability over time at pH 7 and 50°C of formulations of CA, 7-ketolithocholic acid, CDCA and DCA in solution with maltodextrin prepared according to Example I. The concentrations of the bile acids were measured by HPLC and the concentration of the bile acid as a percentage of its concentration on day 0 is reported in the column labeled percentage.

Table 1B shows the results of the test of stability over time at pH 10 and 50°C of formulations of CA, 7-ketolithocholic acid, CDCA and DCA in solution with maltodextrin prepared according to Example I.

Table 2 shows results of the test of stability over time at pH 1 and 50°C of formulations of CA, 7-ketolithocholic acid, CDCA and DCA in solution with maltodextrin at pH 1 and 50°C prepared according to Example II.

Table 1A

CA

Day	#1	#2	#3	Average	Percentage
0	0.529	0.530	0.522	0.527	100.0
4	0.460	0.524	0.524	0.502	95.4
7	0.520	0.525	0.547	0.531	100.8
20	0.516	0.576	0.535	0.542	103.0

KLCA

Day	#1	#2	#3	Average	Percentage
0	0.888	0.879	0.874	0.880	100.0
4	0.871	0.887	0.888	0.882	100.2
7	0.897	0.893	0.888	0.893	101.4
20	0.893	0.909	0.894	0.899	102.1

CDCA

Day	#1	#2	#3	Average	Percentage
0	0.572	0.539	0.530	0.547	100.0
4	0.540	0.552	0.576	0.556	101.6
7	0.581	0.588	0.553	0.574	105.0
20	0.565	0.608	0.560	0.578	105.7

DCA

Day	#1	#2	#3	Average	Percentage
0	0.499	0.491	0.489	0.493	100.0
4	0.501	0.500	0.474	0.491	99.6
7	0.488	0.487	0.484	0.486	98.6
20	0.478	0.476	0.472	0.475	96.3

Table 1B

CA

Day	#1	#2	#3	Average	Percentage
0	0.534	0.524	0.490	0.516	100.0
4	0.501	0.509	0.524	0.511	99.1
7	0.552	0.518	0.533	0.534	103.6
20	0.535	0.563	0.548	0.549	106.4

KLCA

Day	#1	#2	#3	Average	Percentage
0	0.879	0.874	0.857	0.870	100.0
4	0.870	0.873	0.880	0.874	100.5
7	0.893	0.876	0.882	0.884	101.5
20	0.887	0.893	0.887	0.889	102.2

CDCA

Day	#1	#2	#3	Average	Percentage
0	0.541	0.532	0.495	0.522	100.0
4	0.511	0.519	0.538	0.523	100.0
7	0.564	0.527	0.540	0.544	104.1
20	0.556	0.569	0.558	0.561	107.4

DCA

Day	#1	#2	#3	Average	Percentage
0	0.491	0.488	0.471	0.483	100.0
4	0.493	0.487	0.472	0.484	100.2
7	0.479	0.488	0.479	0.482	99.7
20	0.468	0.478	0.479	0.475	98.3

Table 2

CA

Day	#1	#2	#3	Average	Percentage
0	0.516	0.509	0.503	0.509	100.0
4	0.453	0.453	0.466	0.457	89.8
7	0.434	0.426	0.468	0.443	86.9
20	0.207	--	0.206	0.207	40.6

KLCA

Day	#1	#2	#3	Average	Percentage
0	0.883	0.877	0.869	0.876	100.0
4	0.870	0.866	0.847	0.861	98.3
7	0.848	0.844	0.843	0.845	96.4
20	0.661	--	0.651	0.656	74.9

CDCA

Day	#1	#2	#3	Average	Percentage
0	0.560	0.528	0.513	0.534	100.0
4	0.488	0.510	0.519	0.506	94.7
7	0.460	0.469	0.463	0.464	87.0
20	0.169	--	0.154	0.161	30.2

Example III

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH tested.

Soluble UDCA	200 mg (100 mg-2000 mg as free base)
Minimal quantity of maltodextrin	approx. 5 g (approx.1.25 g-50 g)
Preservatives	q.s.
Flavoring agent	q.s.
Sweetener	q.s.
Purified water to	100 mL

80 mL of an aqueous solution in which soluble UDCA is dissolved was prepared, and then, maltodextrin was added into the clear solution with agitating at room temperature. Into the resulting clear solution, sweetener, preservatives and flavoring agents were added in quantities suitable for a pharmaceutical formulation. Purified water was added to adjust the total volume to be 100 mL.

In these formulas, the aqueous solution dosage forms of various concentrations of ursodeoxycholic acid (or its salts) with its corresponding minimal quantity or more of aqueous soluble starch conversion products (for example, maltodextrin, liquid glucose, dried powder of liquid glucose (commercial corn syrup solid), dextran, or soluble starch).

Figure 6 is an NMR spectrum of UDCA illustrating that UDCA, when in a composition prepared according to Example III, is absolutely free UDCA. That is, the

carboxylic acid of UDCA at C-24 is the free form (R-COOH) and two hydroxy group at C-3 and C-7 are in the free form (R-OH)

In addition, the HPLC profile of UDCA in a composition prepared according to Example III (Figure 7) is similar to the profile of of UDCA dissolved in methanol (Figure 8). This data shows that there is no UDCA-complex compound. There is only free UDCA. The UDCA standard solution was prepared by dissolving 100 mg UDCA in 100 mL of methanol. A mixture of acetonitrile (51), water (49), and acetic acid (1) was used as the mobile phase.

Example IV

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH within the selected, desired range of pH values.

Soluble UDCA	0.2g (1g-2g as free acid)
Maltodextrin	5g (35g-50g)
Branched chained amino acid (e.g. leucine, isoleucine, valine)	15g (5g-15g as free base)
Sweetener	q.s.
Flavoring agent	q.s.
Purified water to	100 mL

85 mL of the aqueous solution in which soluble UDCA is dissolved was prepared, and then maltodextrin was added into the clear solution. Into the resulting clear solution, branched amino acids were added with adjusting the pH (4-7) with agitation and then sweetener, preservatives, and flavoring agent were added. Purified water was added to adjust the total volume to be 100 mL.

Based on these formulations, the aqueous solution dosage forms of various concentrations of ursodeoxycholic acid (or its salt) and its corresponding minimal quantity or more of maltodextrin, liquid glucose, dried powder of liquid glucose (commercial corn syrup) or dextran) with various quantities of branched amino acid (total amount of leucine, isoleucine and valine) were prepared.

Tables 3A to 3F show stability test results over time of formulation prepared with amino acids according to Example IV. All stability tests were conducted at

50° C. Stability test results at pH 1 (Table 3A), pH 3 (Table 3B), pH 5 (Table 3C), pH 7 (Table 3D), pH 9 (Table 3E), and pH 10 (Table 3F) are shown.

Table 3A : Stability of UDCA solution according to Example IV at pH1, 50°C

Ile

Day	#1	#2	#3	Average	Percentage
0	0.261	0.236	0.249	0.248	100.0
1	0.256	0.275	0.251	0.261	105.0
2	0.268	0.263	0.251	0.260	104.9
6	0.295	0.268	0.291	0.285	114.6
7	0.249	0.254	0.267	0.257	103.4
8	0.253	0.243	0.240	0.245	98.8
9	0.263	0.268	0.263	0.265	106.6

Leu

Day	#1	#2	#3	Average	Percentage
0	0.485	0.428	0.470	0.461	100.0
1	0.470	0.477	0.456	0.468	101.5
2	0.485	0.481	0.460	0.475	103.1
6	0.553	0.510	0.529	0.531	115.1
7	0.478	0.473	0.513	0.488	105.8
8	0.474	0.454	0.511	0.480	104.0
9	0.483	0.485	0.476	0.481	104.4

Val

Day	#1	#2	#3	Average	Percentage
0	0.506	0.448	0.460	0.471	100.0
1	0.438	0.458	0.471	0.456	96.7
2	0.479	0.485	0.513	0.492	104.5
6	0.505	0.536	0.549	0.530	112.4
7	0.494	0.465	0.496	0.485	102.9
8	0.488	0.491	0.459	0.479	101.7
9	0.479	0.496	0.490	0.488	103.6

Sol

Day	#1	#2	#3	Average	Percentage
0	0.319	0.315	0.322	0.319	100.0
1	0.332	0.344	0.351	0.342	107.4
2	0.371	0.339	0.403	0.371	116.4
6	0.396	0.409	0.411	0.405	127.2
7	0.365	0.351	0.381	0.366	114.7
8	0.409	0.365	0.331	0.368	115.6
9	0.338	0.391	0.374	0.368	115.4

UDCA

Day	#1	#2	#3	Average	Percentage
0	0.388	0.387	0.389	0.388	100.0
1	0.367	0.370	0.366	0.368	94.8
2	0.374	0.388	0.388	0.383	98.9
6	0.371	0.380	0.382	0.377	97.3
7	0.378	0.376	0.379	0.378	97.4
8	0.374	0.382	0.384	0.380	97.9
9	0.370	0.367	0.370	0.369	95.1

Table 3B : Stability of UDCA solution according to Example IV at pH 3, 50°C

Ile

Day	#1	#2	#3	Average	Percentage
0	0.261	0.254	0.253	0.256	100.0
1	0.266	0.268	0.261	0.265	103.3
2	0.273	0.243	0.247	0.254	99.3
6	0.296	0.306	0.300	0.301	117.4
7	0.247	0.265	0.257	0.256	100.0
8	0.250	0.247	0.247	0.248	96.7
13	0.285	0.240	0.250	0.258	100.9

Leu

Day	#1	#2	#3	Average	Percentage
0	0.495	0.465	0.452	0.471	100.0
1	0.489	0.480	0.470	0.480	101.9
2	0.495	0.472	0.481	0.483	102.6
6	0.522	0.532	0.556	0.537	114.0
7	0.492	0.482	0.491	0.488	103.7
8	0.543	0.515	0.495	0.517	109.9
13	0.512	0.496	0.543	0.517	109.8

Val

Day	#1	#2	#3	Average	Percentage
0	0.485	0.491	0.498	0.491	100.0
1	0.467	0.481	0.446	0.465	94.6
2	0.510	0.493	0.527	0.510	103.8
6	0.527	0.491	0.553	0.524	106.6
7	0.485	0.481	0.468	0.478	97.3
8	0.490	0.491	0.544	0.508	103.5
13	0.519	0.498	0.517	0.511	104.1

Sol

Day	#1	#2	#3	Average	Percentage
0	0.343	0.355	0.370	0.356	100.0
1	0.340	0.350	0.316	0.335	94.2
2	0.383	0.371	0.400	0.385	108.0
6	0.378	0.341	0.416	0.378	106.3
7	0.355	0.381	0.315	0.350	98.4
8	0.343	0.350	0.395	0.363	101.9
13	0.377	0.382	0.423	0.394	110.7

UDCA

Day	#1	#2	#3	Average	Percentage
0	0.395	0.396	0.393	0.395	100.0
1	0.396	0.401	0.392	0.396	100.4
2	0.427	0.421	0.416	0.421	106.8
6	0.407	0.408	0.402	0.405	102.7
7	0.412	0.409	0.411	0.411	104.1
8	0.415	0.418	0.408	0.414	104.9
13	0.415	0.412	0.416	0.414	105.0

Table 3C : Stability of UDCA solution according to Example IV at pH 5, 50°C

Ile

Day	#1	#2	#3	Average	Percentage
0	0.285	0.258	0.295	0.279	100.0
3	0.280	0.275	0.275	0.277	99.0
6	0.285	0.273	0.270	0.276	98.7
10	0.274	0.276	0.276	0.275	98.4
13	0.273	0.287	0.278	0.279	100.0
17	0.278	0.276	0.270	0.275	98.3
20	0.261	0.275	0.261	0.266	95.0
24	0.267	0.274	0.292	0.277	99.3

Leu

Day	#1	#2	#3	Average	Percentage
0	0.495	0.467	0.535	0.499	100.0
3	0.510	0.495	0.494	0.500	100.1
6	0.489	0.479	0.484	0.484	97.0
10	0.486	0.490	0.499	0.492	98.5
13	0.492	0.509	0.508	0.503	100.8
17	0.514	0.508	0.504	0.509	100.9
20	0.499	0.500	0.499	0.499	101.1
24	0.488	0.509	0.528	0.508	101.9

Val

Day	#1	#2	#3	Average	Percentage
0	0.483	0.498	0.481	0.487	100.0
3	0.492	0.494	0.526	0.504	103.4
6	0.459	0.475	0.481	0.472	96.8
10	0.500	0.436	0.480	0.472	96.9
13	0.464	0.451	0.474	0.463	95.0
17	0.407	0.491	0.462	0.453	93.0
20	0.471	0.512	0.477	0.487	99.9
24	0.471	0.476	0.458	0.468	96.1

Sol

Day	#1	#2	#3	Average	Percentage
0	0.341	0.351	0.360	0.351	100.0
3	0.342	0.386	0.371	0.366	104.5
6	0.316	0.321	0.342	0.326	93.1
10	0.341	0.299	0.335	0.325	92.7
13	0.355	0.326	0.350	0.344	98.0
17	0.334	0.376	0.353	0.354	101.0
20	0.347	0.398	0.394	0.380	108.3
24	0.416	0.353	0.378	0.382	109.0

UDCA

Day	#1	#2	#3	Average	Percentage
0	0.407	0.404	0.404	0.405	100.0
3	0.409	0.402	0.403	0.405	99.9
6	0.410	0.403	0.409	0.407	100.6
10	0.404	0.405	0.407	0.405	100.1
13	0.408	0.403	0.395	0.402	99.3
17	0.411	0.402	0.404	0.406	100.2
20	0.405	0.394	0.396	0.398	98.4
24	0.399	0.408	0.406	0.404	99.9

Table 3D : Stability of UDCA solution according to Example IV at pH 7, 50°C

Ile

Day	#1	#2	#3	Average	Percentage
0	0.296	0.289	0.281	0.289	100.0
5	0.300	0.282	0.281	0.288	99.7
8	0.277	0.282	0.268	0.276	95.5
12	0.273	0.278	0.278	0.277	95.8
15	0.271	0.273	0.266	0.270	93.5
19	0.294	0.285	0.281	0.287	99.3

Leu

Day	#1	#2	#3	Average	Percentage
0	0.519	0.513	0.495	0.509	100.0
5	0.499	0.499	0.498	0.498	97.9
8	0.498	0.513	0.480	0.497	97.7
12	0.508	0.516	0.515	0.513	100.9
15	0.503	0.505	0.499	0.502	98.7
19	0.521	0.509	0.516	0.515	101.3

Val

Day	#1	#2	#3	Average	Percentage
0	0.483	0.530	0.525	0.513	100.0
5	0.502	0.447	0.499	0.483	94.1
8	0.488	0.498	0.493	0.493	96.2
12	0.490	0.469	0.443	0.467	91.2
15	0.492	0.541	0.442	0.492	95.9
19	0.458	0.500	0.482	0.480	93.6

Sol

Day	#1	#2	#3	Average	Percentage
0	0.333	0.352	0.363	0.349	100.0
5	0.344	0.309	0.349	0.334	95.6
8	0.334	0.379	0.377	0.363	104.0
12	0.345	0.344	0.317	0.335	96.0
15	0.286	0.406	0.321	0.338	96.7
19	0.338	0.416	0.351	0.368	105.4

UDCA

Day	#1	#2	#3	Average	Percentage
0	0.427	0.416	0.428	0.424	100.0
5	0.406	0.427	0.432	0.422	99.4
8	0.419	0.408	0.417	0.414	97.7
12	0.414	0.418	0.419	0.417	98.4
15	0.413	0.418	0.409	0.414	97.5
19	0.429	0.421	0.424	0.425	100.1

Table 3E : Stability of UDCA solution according to Example IV at pH 9, 50°C

Ile

Day	#1	#2	#3	Average	Percentage
0	0.291	0.286	0.282	0.286	100.0
3	0.266	0.273	0.282	0.273	95.6
6	0.277	0.274	0.272	0.274	95.9
10	0.243	0.245	0.295	0.261	91.2
13	0.246	0.269	0.236	0.250	87.4
17	0.275	0.280	0.245	0.267	93.1

Leu

Day	#1	#2	#3	Average	Percentage
0	0.509	0.513	0.511	0.511	100.0
3	0.485	0.487	0.492	0.488	95.5
6	0.495	0.496	0.492	0.494	96.8
10	0.470	0.467	0.528	0.488	95.6
13	0.461	0.491	0.450	0.467	91.5
17	0.468	0.516	0.500	0.495	96.9

Val

Day	#1	#2	#3	Average	Percentage
0	0.508	0.476	0.484	0.489	100.0
3	0.463	0.487	0.485	0.478	97.8
6	0.493	0.473	0.495	0.487	99.5
10	0.441	0.428	0.471	0.447	91.3
13	0.467	0.483	0.537	0.496	101.3
17	0.499	0.495	0.501	0.498	101.8

Sol

Day	#1	#2	#3	Average	Percentage
0	0.341	0.316	0.328	0.328	100.0
3	0.297	0.317	0.317	0.310	94.5
6	0.313	0.291	0.314	0.306	93.2
10	0.268	0.253	0.324	0.282	85.8
13	0.270	0.266	0.334	0.290	88.3
17	0.337	0.329	0.317	0.328	99.8

UDCA

Day	#1	#2	#3	Average	Percentage
0	0.389	0.385	0.389	0.388	100.0
3	0.405	0.400	0.394	0.400	103.2
6	0.427	0.411	0.416	0.418	107.9
10	0.420	0.418	0.450	0.429	110.8
13	0.465	0.434	0.441	0.447	115.3
17	0.454	0.457	0.413	0.441	113.9

Table 3F : Stability of UDCA solution according to Example IV at pH 10, 50°C

Ile

Day	#1	#2	#3	Average	Percentage
0	0.292	0.282	0.287	0.287	100.0
2	0.253	0.237	0.239	0.243	84.7
5	0.221	0.212	0.221	0.218	76.0
7	0.219	0.215	0.207	0.214	74.5
9	0.206	0.192	0.207	0.202	70.2

Leu

Day	#1	#2	#3	Average	Percentage
0	0.507	0.495	0.509	0.504	100.0
2	0.462	0.442	0.442	0.449	89.1
5	0.429	0.428	0.427	0.428	85.0
7	0.410	0.417	0.414	0.414	82.1
9	0.417	0.377	0.418	0.404	80.2

Val

Day	#1	#2	#3	Average	Percentage
0	0.480	0.506	0.471	0.486	100.0
2	0.536	0.478	0.504	0.506	104.2
5	0.371	0.445	0.400	0.405	83.5
7	0.384	0.384	0.424	0.397	81.8
9	0.389	0.354	0.362	0.368	75.8

Sol

Day	#1	#2	#3	Average	Percentage
0	0.368	0.376	0.331	0.358	100.0
2	0.284	0.257	0.266	0.269	75.1
5	0.053	0.217	0.192	0.154	43.0
7	0.042	0.026	0.156	0.075	20.8
9	0.033	0.019	0.023	0.025	7.0

UDCA

Day	#1	#2	#3	Average	Percentage
0	0.416	0.402	0.406	0.408	100.0
2	0.402	0.397	0.400	0.399	97.9
5	0.425	0.413	0.423	0.420	103.0
7	0.406	0.402	0.408	0.406	99.4
9	0.424	0.426	0.421	0.423	103.8

Example V

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH within the selected, desired pH range. This formulation is based on the known analytical data for pharmaceutical use of bear bile.

Tauro UDCA	7g
Tauro CDCA	1g
Glyco UDCA	0.8g
Glyco CDCA	0.2g
Soluble UDCA	1g (or 3g as free form)
Aqueous soluble starch conversion product	250g.
Sweetener	q.s.
Flavoring agent	q.s.
Purified water to	2.0 L

Soluble UDCA is dissolved in water and then high molecular weight aqueous soluble starch conversion product and water are added. Into the resulting clear solution, Tauro UDCA, Tauro CDCA, Glyco UDCA, Glyco CDCA, sweetener, and flavoring agent were added. Purified water was added to adjust the total volume to be 2.0 L.

Example VI

The aqueous solution dosage forms, according to this invention, containing 200mg of ursodeoxycholic acid (UDCA), were prepared according to the method described in the above-described Example III and were administered to three healthy men having normal body weight after fasting. The hematic levels of UDCA and glyco UDCA were evaluated by means of well known chemical methods. After applying buffered serum to sep-pak column, methanol eluate was derivatized with phenacyl bromide at 80°C for 45 minutes. These phenacyl bromide derivatives were dissolved in acetonitrile in preparation for HPLC. The experimental results of the absorption measured at certain times after dosage administration include the total absorption expressed as the area under the serum concentration-time curve (AUC: µg/mL x hours), the maximum hematic concentration (C_{max}; µg/mL) that has been obtained, and the time (T_{max}; hour) in which said maximum concentration has been obtained. These results are reported in Table 4, Figure 1, and Figure 2.

The experimental pharmacokinetic tests of the aqueous solution dosage forms according to this invention carried out on men show substantial improvement in AUC, C_{max} and T_{max} in comparison with the best results from any dosage forms known presently. The maximum hematic concentration (C_{max}) in Table 4 shows an average of 8.43±1.69 µg/mL which is at least two times higher than that reported for use of enteric coated sodium salt of UDCA preparations and four times higher than that obtained using regular UDCA tablet preparations. Moreover, the time of peak concentration (T_{max}) which is related closely to the rate of absorption of UDCA from the aqueous solution dosage forms is 0.25 hours, at least three times faster than the fastest T_{max} previously known.

Table 4A and Table 4B show plasma concentration of UDCA and GUDCA measured in 3 men over time following on oral administration of the UDCA and GUDCA containing formulations according to Example VI and comparison of results against results of others employing different pharmaceutical formulations of UDCA.

Table 5 shows phamacokinetic parameters of UDCA in human after an oral administration of liquid formulation of UDCA. C_{max} is shown.

Taken together, the data in Tables 4 and 5 and Figures 3 and 4 illustrate the supperiority of formulations of the instant invention over conventional formulations with respect to C_{max} and T_{max}. the instant The inventive solutions were effect without any break-down of the solution system caused by the pH of the environment in the stomach and intestines. The therapeutic potential of bile acid and possibly even added pharmaceuticals may be more fully realized using the forulations of the invention. When the therapeutically active ingredients in aqueous solution forms are not precipitated as solid by acidic gastric juices in the stomach and by the various alkaline pH levels of the intestine, the formulation overcomes as a natural consequence, the scarce bioavailability resulted by the unexpected, undesirable results for the extent and the rate of release by disintegration, dissolution and/or diffusion should be overcome.

Table 4A: Plasma concentration of UDCA and GUDCA after an oral administration of this invention at a dose of 200 mg to three men

	UDCA				GUDCA			
Time(h)	#1	#2	#3	mean	#1	#2	#3	mean
0.25	5.1202	10.9171	9.159	8.43±1.69	0.1419	0.4549	0.3328	0.31±0.09
0.5	4.4528	7.7432	7.4395	6.55±1.05	0.2564	1.2455	0.864	0.79±0.29
1	1.6921	1.546	0.2163	1.15±0.47	0.2162	0.6926	0.2142	0.37±0.16
1.5	0.5256	0.2759	0.168	0.32±0.11	1.1573	0.1929	0.4752	0.61±0.29
2	0.2349	0.2176	0.1227	0.19±0.03	0.4013	0.0312	0.0657	0.17±0.12
3	0.1237	N.D.	0.2074	0.17±0.04	0.5085	0.4303	0.3315	0.42±0.05
5					1,9205	0.0229	1.6311	1.18±0.61
7					0.5328	0.4797	0.91	0.64±0.14
AUC (μg·h/mL)	4.32	6.6	5.47	5.46±0.66	6.26	2.22	4.65	4.38±1.17
C_{max} (μg/mL)	5.21	10.92	9.16	8.43±1.69	1.92	1.25	1.63	1.6
T_{max}(h)	0.25	0.25	0.25	0.25	5	0.5	5	3.5±1.5

Table 4B: Pharmacokinetic parameters of UDCA in human after an oral administration of UDCA (M±S.E.)

	C_{max} (ug/mL)	T_{max} (hr)
Roda et al. (1994)		
UDCA gelatine capsule, 450 mg	2.59	3.8
NaUDC gelatine capsule, 475 mg	3.42	2.4
NaUDC enteric-coated, 475 mg	10	3.4
Nagamatsu et al. (1997)		
UDCA 200mg	1.9±0.25	1.5±0.4
UDCA 400mg	7.09±1.43	0.8±0.2
UDCA in this invention, 200mg	8.43±1.69	0.25

Table 5A : Pharmacokinetic parameter (C_{max}) of UDCA in human after oral administration of a liquid solution containing 600 mg UDCA per day.

Time (min)	Person #1	Person #2	Person #3	Person #4	Person #5	Average	Std Dev
0	0.35	1.63	0.40	0.00	0.71	0.618	0.619
5	2.51	9.79	1.68	2.65	6.26	4.578	3.405
15	12.50	47.46	8.34	11.84	21.83	20.394	15.933
60	9.72	6.46	7.77	9.81	17.25	10.202	4.183
120	3.77	1.71	1.40	1.15	2.81	2.168	1.097
240	0.65	0.93	0.50	0.48	1.30	0.772	0.346

Table 5B : Pharmacokinetic parameter (C_{max}) of UDCA in human after an oral administration of a syrup containing 600 mg UDCA per day.

Time (min)	Person #1	Person #2	Person #3	Person #4	Person #5	Average	Std Dev
0	0.62	0.58	0.38	0.00	0.41	0.398	0.246
5	2.76	2.63	0.83	1.42	2.24	1.976	0.827
15	7.80	4.45	3.54	5.85	14.08	7.144	4.197
60	16.08	20.33	8.76	12.06	17.77	15.000	4.605
120	3.98	4.24	5.09	7.79	3.00	4.820	1.820
240	0.81	0.99	1.47	1.85	1.17	1.258	0.411

Example VII

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH within the selected desired range of pH values.

Soluble UDCA	0.2g(0.05g-2g as free acid)
Dried powder of liquid glucose (Commercial corn syrup solid)	20 g (3g-120g)
Soluble non starch polysaccharide (Guar gum or pectin, etc.)	0.01g(0.001g-0.05g)
Purified water to make	100 mL

85 mL of the aqueous solution in which soluble UDCA is dissolved was prepared, and then the mixture of dried powder of liquid glucose, a high molecular weight aqueous soluble starch conversion product and a soluble non starch polysaccharide (guar gum, pectin, etc.) was added into the clear solution. Purified water was added to adjust the total volume to 100 mL.

Example VIII: Mixture Solution

The formulations of Examples VIII, IX, and X include bismuth sulfate. In each of these examples, solution dosage forms were prepared by adding an amount of an ammonium salt of bismuth sulfate sufficient to provide the indicated amount of bismuth sulfate.

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH within the selected desired range of pH values.

UDCA	5 g
CDCA	5 g
Bismuth citrate	5 g
Corn syrup solid	260 g
Citric acid	q.s.
Purified water to make	1.0 L

The UDCA and CDCA were first dissolved in 1.5 mL of a 1N NaOH solution. Next, to the resulting clear solution were added the bismuth citrate and 150 mL of water. Then, the corn syrup solid was added portion by portion with vigorous agitation. The resulting solution was titrated to pH 4 with citric acid. Purified water was added to adjust the total volume to 1.0 L.

Example IX: UDCA-Syrup (20 g UDCA/L)

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH within the selected desired range of pH values.

UDCA	20 g
1 N NaOH	60 mL
Maltodextrin	700 g
Bismuth citrate	4 g
Citric acid or lactic acid	q.s.
Purified water to make	1.0 L

The UDCA is first dissolved in 60 mL of a 1N NaOH solution. Next, to the resulting clear solution were added the bismuth sulfate and 150 mL of water. Then, the maltodextrin was added portion by portion with vigorous agitation. The resulting solution was titrated to pH 3.5 with citric acid. Purified water was added to adjust the total volume to 1.0 L.

Example X: UDCA-Syrup (20 g UDCA/L)

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH within the selected desired range of pH values.

UDCA	20 g
1 N NaOH	60 mL
Corn syrup solid	1,050 g
Bismuth citrate	4 g
Citric acid or lactic acid	q.s.
Purified water to make	1 L

The UDCA is first dissolved in 60 mL of a 1N NaOH solution. Next, to the resulting clear solution were added the bismuth sulfate and 280 mL of water. Then, 1,050 g of corn syrup solid was added portion by portion with vigorous agitation. The resulting solution was titrated to pH 3.5 with citric acid. Purified water was added to adjust the total volume to 1.0 L.

Example XI: UDCA-Thick Syrup (30 g UDCA/L)

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH within the selected desired range of pH values.

UDCA	30 g
1 N NaOH	90 mL
Maltodextrin	1,050 g
Citric acid or lactic acid	50 g
Purified water to make	1.0 L

The UDCA is first dissolved in 90 mL of a 1N NaOH solution. Next, to the resulting clear solution were added the bismuth sulfate and 250 mL of water. Then, 1,050 g of maltodextrin was added portion by portion with vigorous agitation. The resulting solution was titrated to pH 3 by the addition of 50 g of citric acid. Purified water was added to adjust the total volume to 1.0 L.

Example XII: UDCA-Thick Syrup (30 g UDCA/L)

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH within the selected desired range of pH values.

UDCA	30 g
1 N NaOH	90 mL
Corn syrup solid	1,500 g
Citric acid or lactic acid	50 g
Purified water to make	1.0 L

The UDCA is first dissolved in 90 mL of a 1N NaOH solution. Next, to the resulting clear solution were added the bismuth sulfate and 230 mL of water. Then, 1,500 g of corn syrup solid was added portion by portion with vigorous agitation. The resulting solution was titrated to pH 3 by the addition of 50 g of citric acid. Purified water was added to adjust the total volume to 1.0 L.

Example XIII: UDCA-Paste (45 g UDCA/L)

The formulations of Examples XIII, XIV, XV, and XVI include bismuth citrate. In each of these examples, solution dosage forms were prepared by adding an amount of an ammonium salt of bismuth citrate sufficient to provide the indicated amount of bismuth citrate.

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH within the selected desired range of pH values.

UDCA	45 g
1 N NaOH	135 mL
Maltodextrin	1,575 g
Bismuth citrate	10 g
Citric acid or lactic acid	q.s.
Purified water to make	1.0 L

The UDCA is first dissolved in 135 mL of a 1N NaOH solution. Next, to the resulting clear solution were added the bismuth citrate and 200 mL of water. Then, 1,575 g of maltodextrin was added portion by portion with vigorous agitation. The resulting solution was titrated to pH 3 by the addition of citric acid. Purified water was added to adjust the total volume to 1.0 L.

Five human subjects were provided with dosage forms prepared according to this Example. The results are shown in Tables 5A and 5B and rendered graphically in Figures 3 and 4. A comparrison of the sharp peak of Figure 3 with the broad peak of

Figure 4 indicates that, by adjusting the dosage form, a practitioner may manipulate the bile acid C_{max} and T_{max}.

H. pylori were cultured on Columbia Bood Agar Base (CRAB) media containing a preparation of Example IX. 2 L of CRAB plates were prepared which contained 9.9 g of CRAB, 9.1 g of tryptic soy agar, 50 mL of sheeps blood, vacomycin, amphotericin B, polymixin B, 2 mL of Example IX, and 358 mL distilled water. After 48 or 72 hours of microaerophillic incubation, bateria were fixed using Karnovsky's fixative and embedded in epon. Electron micrographs of *H. pylori* cells are shown in figures Figures 5A to 5C.

Example XIV: UDCA-Paste (45 g UDCA/L)

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH within the selected desired range of pH values.

UDCA	45 g
1 N NaOH	135 mL
Corn syrup solid	2,300 g
Citric acid or lactic acid	50 g
Purified water to make	1.0 L

The UDCA is first dissolved in 135 mL of a 1N NaOH solution. Next, to the resulting clear solution were added the bismuth citrate and 150 mL of water. Then, 2,300 g of corn syrup solid was added portion by portion with vigorous agitation. The resulting solution was titrated to pH 3 by the addition of citric acid. Purified water was added to adjust the total volume to 1.0 L.

Example XV: Mixture solution of UDCA (22 g) and CDCA (3 g)

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH within the selected desired range of pH values.

UDCA	22 g
1 N NaOH	75 mL
CDCA	3 g
Maltodextrin	875 g
Bismuth citrate	4 g
Citric acid or lactic acid	q.s.
Purified water to make	1.0 L

The UDCA and CDCA are first dissolved in 75 mL of a 1N NaOH solution. Next, to the resulting clear solution were added the bismuth citrate and 240 mL of water. Then, 875 g of maltodextrin was added portion by portion with vigorous agitation. The resulting solution was titrated to pH 3 by the addition of citric acid. Purified water was added to adjust the total volume to 1.0 L.

Example XVI: Mixture solution of UDCA (22 g) and CDCA (3 g)

Solution dosage forms that were prepared according to the following guidelines did not show any precipitation at any pH within the selected desired range of pH values.

UDCA	22 g
1 N NaOH	75 mL
CDCA	3 g
Corn syrup solid	1,320 g
Bismuth citrate	4 g
Citric acid or lactic acid	q.s.
Purified water to make	1.0 L

The UDCA and CDCA are first dissolved in 75 mL of a 1N NaOH solution. Next, to the resulting clear solution were added the bismuth citrate and 240 mL of water. Then, 1,320 g of corn syrup solid was added portion by portion with vigorous agitation. The resulting solution was titrated to pH 3 by the addition of citric acid. Purified water was added to adjust the total volume to 1.0 L.

Example XVII

The effect of treating *H. pylori* infected mice with a solution dosage form of the invention was tested. Six week old C57BL/6 female mice were infected by feeding at diet comprising 10^9 CFU/mL *H. pylori*, SS1 strain. The animals consumed this feed twice, one week apart. Subsequently, 0.2 mL of a solution dosage form according to Example VIII was administered to four infected animals once per day for one week. Two

animals were sacrificed one week following administration of the last dose of the inventive solution. The remaining two animals were sacrificed four weeks following administration of the last dose of the inventive solution. Whole stomachs were washed with saline to remove mucosa and debris. A sample of stomach tissue from each animal was subjected to a CLO test using a rapid urease test kit (Delta West, Australia). Each residual stomach was fixed with 10% formalin solution and embedded with paraffin. Sections (4 μm thick) were collected on glass slides and stained with H&E staining solution and Warthin staining solution. Tissue was evaluated for pathological status by conventional light microscopy.

The results, summarized in Table 6, indicate that the urease test results were negative for mice passed one week after discontinuing administration of the liquid dosage form, and *H. pylori* was not seen in Warthin examination. Of the other two mice, one showed a negative urease test and no *H. pylori* were seen by Warthin examination. The other, however, yielded a positive urease test although only a few *H. pylori* were seen in Warthin examination.

Table 6

Weeks After Treatment	Animal	Urease Test	Warthin Examination
1	1	Negative	No *H. pylori*
1	2	Negative	No *H. pylori*
4	3	Negative	No *H. pylori*
4	4	Positive	A few *H. pylori*

Example XVIII

Assays for growth of *H. pylori* on media containing UDCA, bismuth citrate or both UDCA and bismuth citrate were performed. For these assays the following media was used:

000112B-1 having a pH of 4.0 and comprising 525 g/L maltodextrin and 15 g/L UDCA.

OSABY having a pH of 3.7 and comprising 1 kg/L corn syrup solid and 6 g/L bismuth citrate.

Three assays were performed to assess the growth capacity of *H. pylori* in the presence of UDCA, bismuth or both wherein the pH, concentration, and length of exposure was varied.

1. *Helicobacter pylori* was suspended in physiological saline to give about 10^9 organisms per milliliter. 50 µL of this innoculum was transferred to tubes containing 1 mL of citrate-phosphate buffer at pH 3.0, 4.0, and 4.5. Paired tubes were prepared with and without 6 mM Urea. Following a 30 minute room temperature incubation, the suspensions were subcultured on agar plates containing 000112B-1 using a 1 µL loop. Plates were incubated microaerophilically at 37° C for 72 hours. This procedure is illustrated in Figure 9.

As shown in Table 7, *H. pylori* grew poorly on pH 3 and pH 4 control media. Table 7 further shows that *H. pylori* does not grow on pH 3 and pH 4 media containing UDCA. The designations "3 ml", "4 ml" and "5 ml" refer to the total volume of 000112B-1 media per plate. "PBS" is phosphate buffered saline at pH 7.0.

Table 7

Plate	pH	Urea	Urease Test			
			1-2 sec.	10 min.	2 hr.	20 hr.
Control	3.0	Yes	FO	FO	O	O
		No	FO	FO	O	O
	4.0	Yes	FO	FO	O	O
		No	FO	FO	O	P
	4.5	Yes	FP	FP	P	P
		No	FO	O	FP	P
	PBS	Yes	O	FP	P	P
		No	O	FP	P	P
000112B-1 (3 mL)	3.0	Yes	Y	Y	Y	Y
		No	Y	Y	Y	Y
	4.0	Yes	Y	Y	FO	O
		No	Y	Y	Y	FO
	4.5	Yes	FP	FP	P	P
		No	FP	FP	P	P
000112B-1 (4 mL)	3.0	Yes	Y	Y	Y	Y
		No	Y	Y	Y	Y
	4.0	Yes	Y	Y	Y	FO
		No	Y	Y	FO	FO
	4.5	Yes	FP	FP	P	P
		No	FP	FP	FP	P
000112B-1 (5 mL)	3.0	Yes	Y	Y	Y	Y
		No	Y	Y	Y	Y
	4.0	Yes	Y	Y	FO	FO
		No	Y	Y	Y	Y
	4.5	Yes	FP	FP	P	P
		No	FP	FP	P	P

Key

	Y	FO	O	FP	P
Color	Yellow	Faint Orange	Orange	Faint Pink	Pink
Helicobacter	None	Very Rare	Rare	Exist	Many

2. *Helicobacter pylori* was suspended in physiological saline to give about 10^9 organisms per milliliter. 50 μL of this innoculum was transferred to tubes containing 1 mL of citrate-phosphate buffer at various concentrations of plating media such as 1/10, 1/30, 1/50, 1/100, 1/200, 1/500, 1/800, 1/1000, 1/2000. All tubes were prepared with 6 mM Urea. Following a 30 minute room temperature incubation, the suspensions were subcultured on agar plates using a 1 μL loop. These plates were substantially free of bismuth and bile acids. Plates were incubated microaerophilically at 37° C for 72 hours. This procedure is illustrated in Figure 10.

Table 8 shows urease test results following 72 hours of growth of *H. pylori* on media prepared with dilutions of UDCA (000112B-1) bismuth citrate (OSABY) or both UDCA and bismuth citrate. Poor growth of *H. pylori* on media containing either UDCA or bismuth citrate was observed (Table 8). Growth of H. pylori was further attenuated when cultured on media containing both UDCA and bismuth citrate (Table 8).

Table 8

Plate	Urease Test			
	Immediately	10 min.	30 min.	60 min.
000112B-1				
Control	P	P	P	P
1/10	Y	FP	P	P
1/30	Y	FP	P	P
1/50	Y	FP	P	P
1/100	Y	FP	P	P
1/200	Y	FP	P	P
1/500	Y	FP	P	P
1/800	FP	P	P	P
1/1000	FP	P	P	P
1/2000	FP	P	P	P
OSABY				
Control	P	P	P	P
1/10	Y	FP	P	P
1/30	Y	FP	P	P
1/50	Y	FP	P	P
1/100	FP	FP	P	P
1/200	FP	FP	P	P
1/500	FP	FP	P	P
1/800	FP	P	P	P
1/1000	FP	P	P	P
1/2000	FP	P	P	P
000122B-1 + OSABY				
Control	P	P	P	P
1/10	Y	FP	FP	FP
1/50	Y	Y	Y	Y
1/100	Y	Y	Y	Y
1/500	Y	FP	FP	FP
1/1000	Y	FP	P	P

Key

	Y	FO	O	FP	P
Color	Yellow	Faint Orange	Orange	Faint Pink	Pink
Helicobacter	None	Very Rare	Rare	Exist	Many

3. *Helicobacter pylori* was suspended in physiological saline to give about 10^9 organisms per milliliter. 50 μL of this innoculum was transferred to tubes containing 1 mL of citrate-phosphate buffer at various concentrations such as ½, 1/4, and 1/10 for 15 minutes, ½, 1/4, and 1/10 for 30 minutes, and ½, 1/4, and 1/10 for 45 minutes. Paired tubes were innoculated with and without 6 mM Urea. Following a 30 minute room temperature incubation, the suspensions were subcultured on agar plates using a 1 μL loop. These plates were substantially free of bismuth and bile acids. Plates were incubated microaerophilically at 37° C for 72 hours. This procedure is illustrated in Figure 11.

Table 9 shows urease test results following 72 hours of growth of *H. pylori* on media prepared with dilutions of UDCA (000112B-1) bismuth citrate (OSABY) or both UDCA and bismuth citrate. As indicated, longer exposure times increased the adverse effect of the solutions on *H. pylori*.

Table 9

Dilution	Incubation Time (min.)	Urease Test (min.)				
		1	30	60	120	240
000112B-1						
Control		P	P	P	P	P
½	15	Y	Y	Y	Y	Y
	30	Y	Y	Y	Y	Y
	45	Y	Y	Y	Y	Y
1/4	15	Y	FO	FP	P	P
	30	Y	Y	FO	FO	FO
	45	Y	Y	Y	Y	Y
1/10	15	Y	FO	FO	FO	FO
	30	Y	FO	FO	O	P
	45	Y	Y	Y	FO	FO
OSABY						
Control		P	P	P	P	P
½	15	Y	Y	Y	Y	Y
	30	Y	Y	Y	Y	Y
	45	Y	Y	Y	Y	Y
1/4	15	Y	Y	Y	Y	Y
	30	Y	Y	Y	Y	Y
	45	Y	Y	Y	Y	Y
1/10	15	Y	FO	FO	FO	P
	30	Y	Y	Y	Y	Y
	45	Y	Y	Y	Y	Y
000122B-1 + OSABY						
Control		P	P	P	P	P
½	15	Y	Y	Y	Y	Y
	30	Y	Y	Y	Y	Y
	45	Y	Y	Y	Y	Y
1/4	15	Y	Y	Y	Y	Y
	30	Y	Y	Y	Y	Y
	45	Y	Y	Y	Y	Y
1/10	15	Y	Y	Y	FO	FO
	30	Y	FO	FO	FO	P
	45	Y	Y	Y	Y	Y